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TESI DI LAUREA

How to treat Splenic Marginal Zone Lymphoma (SMZL) in patients unfit for surgery or more aggressive therapies.

CANDIDATO:

Claudia Rossi

RELATORI:

Chiar.mo Prof. Mario Petrini

Dott.ssa Giulia Cervetti

Index:

- **Statistical analyses**
- **Results**
- **Discussion**
- **References**

Malignant Lymphomas:

The term '*malignant lymphoma'* embraces all neoplastic diseases that originates in the lymph nodes or extranodal lymphatic tissue. They comprise Hodgkin lymphoma, which is relatively uniform in histology, and the large heterogeneous category known as the non-Hodgkin lymphomas, which vary from highly proliferating and rapidly fatal disorders to indolent (although often incurable) malignancies that may be well tolerated for 10 to 20 years or more. It has been known for many years that non-Hodgkin lymphomas represent monoclonal expansions of B or T cells or natural killer cells (NK). Evidence of this comes from both expression of a single type of Immunoglobulin (Ig) on the cell surface and/or within the cytoplasm and also from studies of Ig or T-cell receptor gene rearrangement. It's possible to find a normal counterpart for many types of non-Hodgkin lymphoma. The large number of these diseases reflects the rich diversity of much of the maturation stages and sub-populations

of reacting human lymphoid cells. The Reed-Sternberg cells characteristic of a Hodgkin lymphoma are also clonal in origin, deriving from B cells.

Sometimes the difference between lymphomas, in which lymph node, spleen, or other solid tumor is present, and lymphoid leukemias (acute and chronic), with dominant bone marrow disease, is imprecise because lymphomas can be leukemic and leukemias can be lymphomatous (e.g., they can manifest as solid tumor deposits). A single lymphoproliferative disease can be categorized as two clinical manifestations, for example, chronic lymphocytic leukemia and small lymphocytic lymphoma merge into each other, their cell phenotypes and genotypes being identical. Also lymphoblastic lymphomas of precursor B or T cells are now classified with B-cells lineage acute lymphoblastic leukemia (ALL) or T-cell ALL, respectively, and treated as such.

The **frequencies** of some types of non-Hodgkin lymphoma vary markedly between the different parts of the world. For example, two lymphoma categories that are common in Western countries, Hodgkin lymphoma and follicular lymphoma, are much rarer in Eastern and less developed countries, whereas large B-cell lymphoma and T-cell neoplasm are more frequent in the latter areas. Some subtypes of non-Hodgkin lymphoma that are only rarely seen in Western countries are found at much higher frequency elsewhere, and this may be partly accounted for by local patterns of exposure to viruses and other pathogens. In each of these instances the infectious agent presumably provides a stimulating effect on lymphoid cell growth, but how this interacts with other cellular mechanisms to induce neoplastic transformation is unclear.

The **molecular etiology** of lymphomas has been shown by the study of chromosome alterations, and in many instances the consequences of these

alterations have been identified at the DNA level. Other diseases (e.g., mantle cell lymphoma, ALK positive, and anaplastic large cell lymphoma) are defined on the basis of genetic abnormality. In the past many large cell non-Hodgkin lymphomas were referred to as 'histiocytic', but it's now evident that the vast majority of neoplasm arising from the monocyte-phagocyte system manifest as leukemias. [1]

The **classification** of the lymphomas has undergone significant new evaluations over the past 50 years. These changes have resulted from insights gained through the application of molecular and immunologic techniques and a better comprehension of the clinical aspects of lymphoma through advances in diagnosis, staging and treatment.

World Health Organization Classification of Lymphoid Tumors*

B-Cell Neoplasms

B-cell lymphoblastic leukemia/lymphoma
Chronic lymphocytic leukemia/small lymphocytic lymphoma
B-cell prolymphocytic leukemia
Lymphoplasmacytic lymphoma
Mantle cell lymphoma
Follicular lymphoma
Primary cutaneous follicle center lymphoma
Marginal-zone B-cell lymphoma of mucosa-associated lymphoid tissue (MALT)-type
Nodal marginal-zone B-cell lymphoma
Splenic marginal-zone B-cell lymphoma
Hairy cell leukemia
Diffuse large B-cell lymphomas
  Subtypes: mediastinal (thymic), T-cell/ histiocyte-rich large B-cell lymphoma, intravascular, primary effusion lymphoma, plasmablastic, ALK$^+$ large B-cell lymphoma, primary cutaneous diffuse large B-cell lymphoma, leg-type.
Burkitt lymphoma
Plasmacytoma
Plasma cell myeloma

T-Cell Neoplasms

T-cell lymphoblastic leukemia/lymphoma
T-cell prolymphocytic leukemia
T-cell large granular lymphocytic leukemia
Aggressive NK-cell leukemia
Hepatosplenic T-cell lymphoma
Extranodal NK- and T-cell lymphoma, nasal type
Angioimmunoblastic T-cell lymphoma
Peripheral T-cell lymphoma (unspecified)
Adult T-cell leukemia/lymphoma
Anaplastic large cell lymphoma, ALK-positive
Anaplastic large cell lymphoma, ALK-negative
Enteropathy-associated intestinal T-cell lymphoma
Subcutaneous panniculitis-like T-cell lymphoma
Primary cutaneous gamma-delta T-cell lymphoma
Primary cutaneous CD30$^+$ T-cell lymphoproliferative disorders
Lymphomatoid papulosis
Primary cutaneous anaplastic large cell lymphoma
Mycosis fungoides
Sezary syndrome

Hodgkin Lymphoma

Nodular lymphocyte-predominant Hodgkin lymphoma
Classical Hodgkin lymphoma
Hodgkin lymphoma, nodular sclerosis
Classical Hodgkin lymphoma, lymphocyte-rich
Hodgkin lymphoma, mixed cellularity
Hodgkin lymphoma, lymphocytic depletion

*Includes updates proposed in the WHO-EORTC classification of cutaneous lymphomas (2005).[109]

Classifying non-Hodgkin lymphoma (NHL) can be quite confusing because there are so many types and because several different systems have been used. The most recent system is the *World Health Organization (WHO)* classification (2008).

WHO classification represents a significant achievement in terms of cooperation among pathologists, hematologists, and oncologists. Moreover, it recognizes, as in the REAL classification, that any classification system is an evolving process and should incorporate new data resulting from recent technologic advances in the field of hemopathology and is subjected to periodical review and revisions. [2]

The WHO system groups lymphomas based on their histological patterns, the chromosome features of the malignant cells, and the presence of certain proteins on the surface of the cells. (Older systems classified lymphomas only by how the cells looked under the microscope). The more common types of lymphoma are listed below.

Mature B-cell neoplasms

- Chronic lymphocytic leukemia/small lymphocytic lymphoma
- B-cell prolymphocytic leukemia
- Hairy cell leukemia
- Splenic marginal zone lymphoma
    - Splenic lymphoma/leukemia, unclassifiable
    - Splenic diffuse red pulp small B-cell lymphoma*
    - Hairy cell leukemia-variant*

- Lymphoplasmacytic lymphoma
    - Waldenström macroglobulinemia
- Heavy chain diseases
    - Alpha heavy chain disease
    - Gamma heavy chain disease
    - Mu heavy chain disease Plasma cell myeloma
- Solitary plasmacytoma of bone
- Extraosseous plasmacytoma
- Extranodal marginal zone B-cell lymphoma of mucosa-associated lymphoid tissue (MALT lymphoma)
- Nodal marginal zone B-cell lymphoma (MZL)
    - Pediatric type nodal MZL
- Follicular lymphoma
    - Pediatric type follicular lymphoma
- Primary cutaneous follicle center lymphoma
- Mantle cell lymphoma
- Diffuse large B-cell lymphoma (DLBCL), not otherwise specified
    - T cell/histiocyte rich large B-cell lymphoma
    - DLBCL associated with chronic inflammation
    - Epstein-Barr virus (EBV)+ DLBCL of the elderly
- Lymphomatoid granulomatosis
- Primary mediastinal (thymic) large B-cell lymphoma
- Intravascular large B-cell lymphoma
- Primary cutaneous DLBCL, leg type
- ALK+ large B-cell lymphoma

- Plasmablastic lymphoma
- Primary effusion lymphoma
- Large B-cell lymphoma arising in HHV8-associated multicentric Castleman disease
- Burkitt lymphoma
- B-cell lymphoma, unclassifiable, with features intermediate between diffuse large B-cell lymphoma and Burkitt lymphoma
- B-cell lymphoma, unclassifiable, with features intermediate between diffuse large B-cell lymphoma and classical Hodgkin lymphoma
- Hodgkin Lymphoma
- Nodular lymphocyte-predominant Hodgkin lymphoma
- Classical Hodgkin lymphoma
    - Nodular sclerosis classical Hodgkin lymphoma
    - Lymphocyte-rich classical Hodgkin lymphoma
    - Mixed cellularity classical Hodgkin lymphoma
    - Lymphocyte-depleted classical Hodgkin lymphoma

Mature T-cell neoplasms

- T-cell prolymphocytic leukemia
- T-cell large granular lymphocytic leukemia
- Chronic lymphoproliferative disorder of NK-cells*
- Aggressive NK cell leukemia Systemic EBV+ T-cell lymphoproliferative disease of childhood (associated with chronic active EBV infection)
- Hydroa vacciniforme-like lymphoma

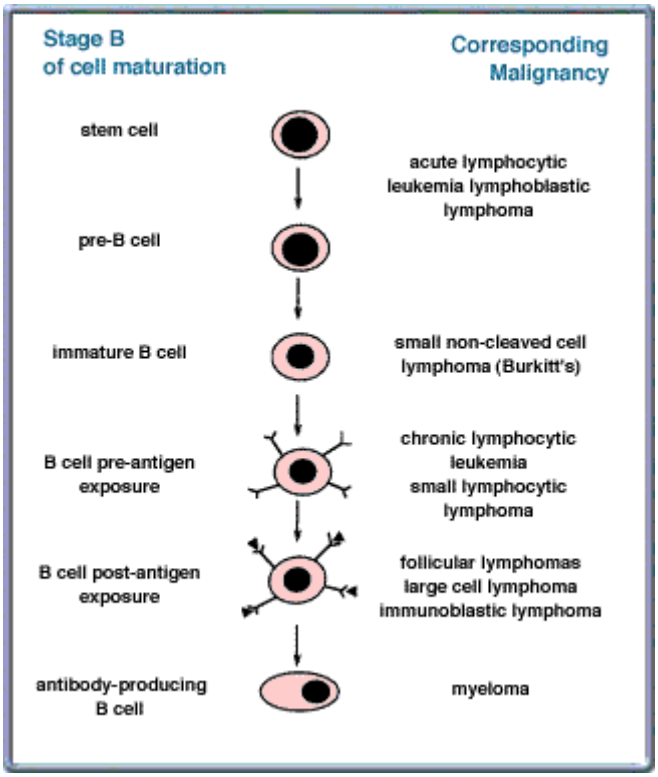
- Adult T-cell leukemia/lymphoma
- Extranodal NK/T cell lymphoma, nasal type
- Enteropathy-associated T-cell lymphoma
- Hepatosplenic T-cell lymphoma
- Subcutaneous panniculitis-like T-cell lymphoma
- Mycosis fungoides Sézary syndrome
- Primary cutaneous CD30+ T-cell lymphoproliferative disorder
    - Lymphomatoid papulosis
    - Primary cutaneous anaplastic large-cell lymphoma
- Primary cutaneous aggressive epidermotropic CD8+ cytotoxic T-cell lymphoma*
- Primary cutaneous gamma-delta T-cell lymphoma
- Primary cutaneous small/medium CD4+ T-cell lymphoma*
- Peripheral T-cell lymphoma, not otherwise specified
- Angioimmunoblastic T-cell lymphoma
- Anaplastic large cell lymphoma (ALCL),
- ALK+ Anaplastic large cell lymphoma (ALCL), ALK–*

*These represent provisional entities or provisional subtypes of other neoplasms.

Additional information about the 2008 WHO classification.

Indolent / Aggressive classification

The following table classifies the lymphoproliferative disorders according to whether they belong to the indolent (slow growing) or aggressive subtype. This includes lymphomas, leukaemias, and myelomas. This is based on the previous REAL/WHO classification system not the current 2008 WHO system.



Indolent lymphoma/leukemia

- A. Follicular lymphoma (follicular small cleaved cell [grade 1], follicular mixed small cleaved and large cell [grade 2], diffuse small cleaved cell)
- B. Chronic lymphocytic leukemia/small lymphocytic lymphoma
- C. Lymphoplasmacytic lymphoma (Waldenstrom's macroglobulinemia)
- D. Extranodal marginal zone B-cell lymphoma (MALT lymphoma
- E. Nodal marginal zone B-cell lymphoma (monocytoid B-cell lymphoma)
- F. Splenic marginal zone lymphoma (splenic lymphoma with villous lymphocytes)
- G. Hairy cell leukemia
- H. Mycosis fungoides/Sezary syndrome

- I. T-cell granular lymphocytic leukemia
- J. Primary cutaneous anaplastic large cell lymphoma/lymphomatoid papulosis (CD30+)
- K. Nodular lymphocyte predominant Hodgkin's lymphoma

Aggressive lymphoma/leukemia

- A. Diffuse large cell lymphoma (includes diffuse mixed cell, diffuse large cell, immunoblastic, T-cell rich large B-cell lymphoma) Distinguish:
    - 1. Mediastinal large B-cell lymphoma
    - 2. Follicular large cell lymphoma (grade 3)
    - 3. Anaplastic large cell lymphoma (CD30+)
    - 4. Extranodal NK/T-cell lymphoma, nasal type
    - 5. Lymphomatoid granulomatosis (angiocentric pulmonary B-cell lymphoma)
    - 6. Angioimmunoblastic T-cell lymphoma
    - 7. Peripheral T-cell lymphoma, unspecified
    - 8. Subcutaneous panniculitis-like T-cell lymphoma
    - 9. Hepatosplenic T-cell lymphoma
    - 10. Enteropathy-type T-cell lymphoma
    - 11. Intravascular large B-cell lymphoma
- B. Burkitt lymphoma/Burkitt cell leukaemia/Burkitt-like lymphoma
- C. Precursor B- or T-cell lymphoblastic lymphoma/leukaemia
- D. Primary CNS lymphoma
- E. Adult T-cell leukaemia/lymphoma (HTLV 1+)

- F. Mantle cell lymphoma
- G. Polymorphic post-transplantation lymphoproliferative disorder (PTLD)
- H. AIDS-related lymphoma
- I. True histiocytic lymphoma
- J. Primary effusion lymphoma
- K. Aggressive NK-cell leukemia/blastic NK-cell lymphoma
- L. B- or T-cell prolymphocytic leukemia

Staging:

Once non-Hodgkin lymphoma is diagnosed, tests are done to determine the stage (extent of spread) of the disease. The treatment and prognosis (outlook) for a patient with non-Hodgkin lymphoma depend in part on the stage of the lymphoma.

Tests used to gather information for staging include:

- Physical exam
- Biopsies of enlarged lymph nodes or other abnormal areas
- Blood tests
- Imaging tests (CT scans; PET; ultrasonography)
- Bone marrow aspiration and biopsy (often but not always done)
- Lumbar puncture (spinal tap – this may not need to be done)

Ann Arbor staging system:

A staging system is a way to summarize the extent of a cancer's spread. The Ann Arbor staging system is most often used to describe the extent of non-Hodgkin lymphoma in adults.

The stages are described by Roman numerals I through IV. Lymphomas that affect an organ outside the lymph system (an extranodal organ) have E added to their stage (for example, stage IIE), while those affecting the spleen have an S added.

Stage I

Either of the following means the disease is stage I:

- The lymphoma is in only 1 lymph node area or lymphoid organ such as the thymus (I).
- The cancer is found only in 1 area of a single organ outside of the lymph system (IE).

Stage II

Either of the following means the disease is stage II:

- The lymphoma is in 2 or more groups of lymph nodes on the same side of (above or below) the diaphragm. For example, this might include nodes in the underarm and neck area but not the combination of underarm and groin nodes (II).
- The lymphoma extends from a single group of lymph node(s) into a nearby organ (IIE). It may also affect other groups of lymph nodes on the same side of the diaphragm.

Stage III

Either of the following means the disease is stage III:

- The lymphoma is found in lymph node areas on both sides of (above and below) the diaphragm.
- The cancer may also have spread into an area or organ next to the lymph nodes (IIIE), into the spleen (IIIS), or both (IIISE).

Stage IV

Either of the following means the disease is stage IV:

- The lymphoma has spread outside the lymph system into an organ that is not right next to an involved node.
- The lymphoma has spread to the bone marrow, liver, brain or spinal cord, or the pleura.

Other modifiers may also be used to describe the lymphoma stage:

Bulky disease

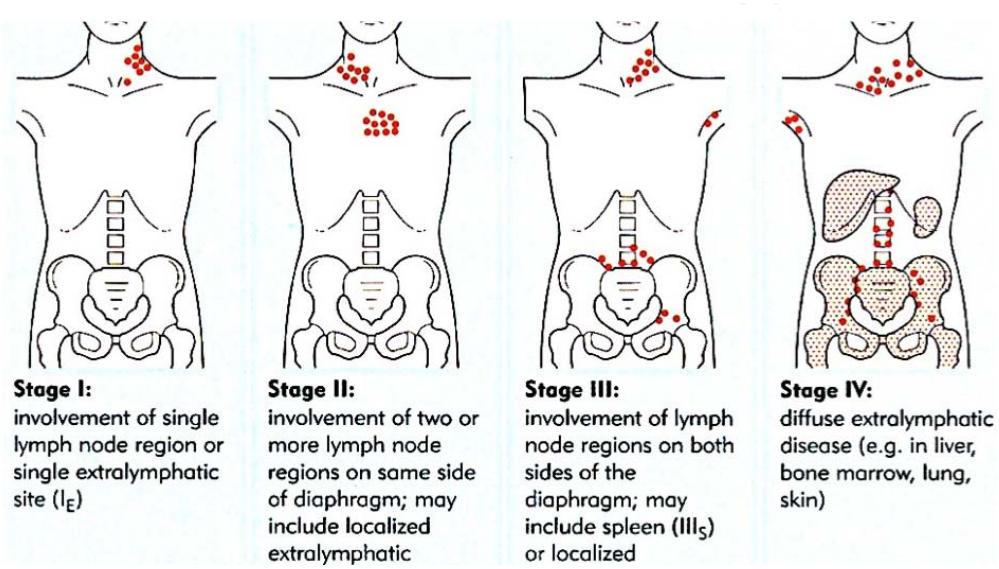
This term is used to describe tumors in the chest that are at least one-third as wide as the chest, or tumors in other areas that are at least 10 centimeters (about 4 inches) across. It is usually designated by adding the letter X to the stage. Bulky disease might need more intensive treatment.

A vs. B

Each stage may also be assigned an A or B. The letter B is added (stage IIIB, for example) if a person has any of the B symptoms listed below:

- Loss of more than 10% of body weight over the previous 6 months (without dieting)
- Unexplained fever of at least 38.5°C
- Drenching night sweats

These symptoms usually mean the disease is more advanced. If a person has any of these, then more intensive treatment is usually recommended. If no B symptoms are present, the letter A is added to the stage.



Indolent Lymphomas:

'Indolent lymphomas' are a group of malignant lymphomas, so called to describe their clinical behavior characterized by a slow growth and spreading and few symptoms for long periods.

Clinical Presentation

Although specific subtypes may be associated with specific presenting features, there are many common features and the majority of patients present with lymphadenopathy. Extranodal disease is common and can affect any organ. The most common sites of extranodal disease include the marrow, skin, gastrointestinal tract, and bone. Symptoms may be nonspecific or related to the site of disease involvement. Many patients with indolent lymphomas are asymptomatic, but some, particularly those with bulky disease, may present with B symptoms defined as fever, drenching sweats, or weight loss of more than 10% of body weight. Patients may present with evidence of bowel obstruction from intra abdominal lymphadenopathy and retroperitoneal disease may manifest as obstructive uropathy. Inguinal disease may cause compression of the venous system with deep venous thrombosis. Central nervous system involvement can occur, but is uncommon in indolent lymphomas. [3]

Diagnosis of Indolent lymphomas

Suggested guidelines for the diagnosis of indolent lymphomas have been outlined by the National Comprehensive Cancer Network and by the European Society for Medical Oncology. Diagnosis should be confirmed by excisional biopsy of an accessible lymph node with review by a hematopathologist. Fine needle aspiration is not indicated, and sufficient material must be obtained for immunophenotyping and genetic studies as required for diagnosis and prognostic markers. If there are not any easily accessible peripheral nodes, computed tomography (CT) or ultrasound guided biopsy are commonly tolerated enough. Where possible, consent should be obtained for the procurement and storage of use of excess tissue from lymph node biopsies at

the time of presentation and at each subsequent relapse of disease for research purposes to investigate the molecular biology of these diseases. Marrow biopsy provides essential information and should be performed routinely. The yield of bilateral marrow biopsy is moderately higher (15%) than that of unilateral biopsy.

Physical examination should include careful examination of all peripheral lymph node groups including the cervical, supraclavicular, axillary and inguinal chains and examination of Waldeyer ring. Abdominal examination should focus on evaluation of any intraabdominal masses, with particular attention paid to detection of enlargement of the liver or spleen. The skin should be carefully examined. Patients may present with pleural or pericardial effusions, although this is less common than in the aggressive lymphomas.

Laboratory investigations should include a complete blood count to evaluate for cytopenias, which may be evidence of marrow infiltration or of autoimmunity. A white blood cell count with differential and examination of the peripheral blood smear may indicate leukemic involvement. Baseline electrolytes including calcium and phosphate, creatinine, and liver function tests are important to determine organ dysfunction that may be related to direct infiltration by lymphoma. Elevation of lactate dehydrogenase is an important prognostic factor and may be a useful indicator of transformation from indolent to aggressive lymphoma. Assessment of immunoglobulin levels and serum electrophoresis are useful, particularly in lymphoplasmacytic lymphoma, to evaluate for monoclonal protein. Cryoglobulins may also be present, particularly in marginal zone lymphoma in association with hepatitis C. A Coombs test and reticulocyte count may be indicated in patients with anemia.

Initial staging workup also includes a CT scan of the chest, abdomen, and pelvis, with particular attention to sites of bulk disease and to the number of involved sites. Gastrointestinal tract workup and biopsy are indicated in mantle cell lymphoma and in patients with mucosa-associated lymphoid tissue (MALT) lymphomas. Liver biopsy may be indicated on the basis of abnormal imaging or laboratory values. [3]

Indolent Non-Hodgkin's lymphomas (iNHLs) encompass the following low-grade histologic subtypes B-cell NHL included in the recent World Health Organization (WHO) **classification** of lymphoid neoplasm published in 2008:

- follicular lymphoma (FL);
- small lymphocytic lymphoma (SLL);
- lymphoplasmacytic lymphoma (LPL), which is defined as Waldenström's macroglobulinemia (WM) when associated with a monoclonal IgM component and bone marrow involvement;
- splenic marginal-zone lymphoma (SMZL);
- primary nodal marginal-zone lymphoma (NMZL);
- marginal-zone lymphoma of mucosa-associated lymphoid tissue (MALT).

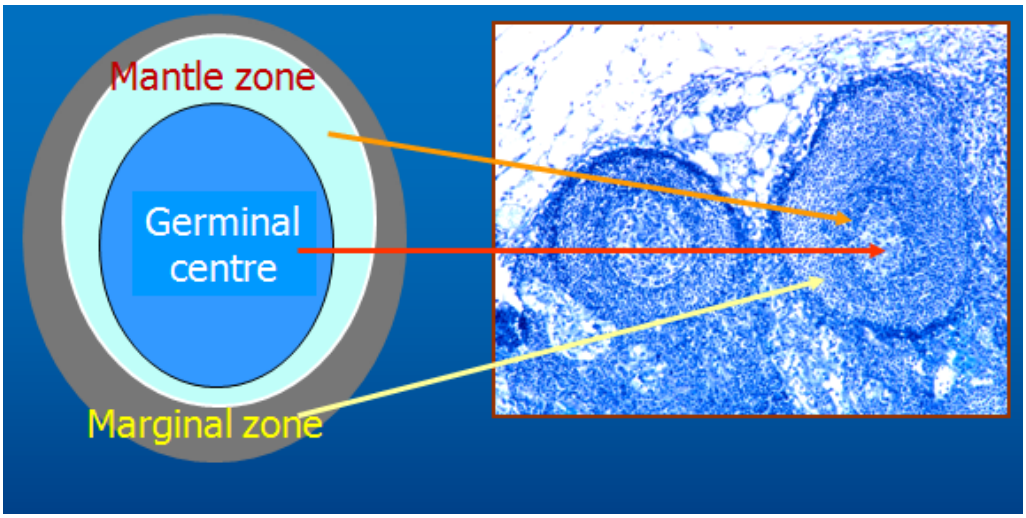
FL is the second most common subtype of NHL, accounting for approximately 25% of newly diagnosed cases of NHL, followed by MALT lymphoma (7% including 'gastric' and 'non gastric' cases), while other subtypes are rather rare, with SLL, LPL, SMZL and NMZL accounting for 3%, 2%, 2% and 1% of NHL patients, respectively. For this reason, given their infrequency with

respect for FL, these entities are frequently grouped altogether under the category of *'indolent non-follicular lymphoma'* (iNFL).

Type	HNL %	median survival (years)
Follicular	25%	10 yrs
Lymphocytic	7%	5-6 yrs
Lymphoplasmacytic	2%	4-5 yrs
Mantle	6%	3 yrs
MALT	7%	6 yrs
Nodal	2%	4-5 yrs
Splenic	**<2%**	8-9 yrs

Marginal Zone Lymphomas

The WHO classification lists three forms of marginal zone lymphomas (MZLs) including extranodal, nodal, and splenic marginal zone lymphomas. However, there are still uncertainties as to whether they represent a homogeneous group of tumors. These lymphomas behave differently than most indolent lymphomas and require different treatment approaches. [3]



The **marginal zone** (MZ) corresponds to the outer part of secondary follicles. It is easily recognizable in the spleen, intraabdominal lymph nodes, and MALT. Most MZ cells express CD19 and CD20 and have the phenotypic profile of memory B cells and are strongly positive for IgM, IgG, or IgA, only a small subpopulation exhibits weak IgD staining. They also express CD21, CD27, and Bcl-2 protein, but are negative for CD5, CD10, CD23, CD43, and CD75. A subset of splenic MZ cells shares phenotypic features with mantle B elements by showing positivity for IgM, IgD, and Ki-B3 and negativity for CD21 and CD27. Most, but not all, splenic MZ B cells show somatic hypermutation of Ig genes contain point mutations of the Ig genes at frequencies found in postfollicular memory B elements. However, a small subset of the same MZ B cells displays a low load of Ig gene point mutations, as usually found in mantle B cells. Therefore, although belonging to the same anatomic compartment, splenic MZ B elements do show a certain phenotypic and molecular variability, the vast majority of them being likely part of the recirculating memory B-cell pool. Interestingly, splenic MZ B cells can bind polysaccharide antigens with one of two results, depending on the follicle microenvironment. First, they can migrate into the germinal centers (GCs) and present the antigen to GC B cells. If follicular dendritic cells have surface Ig that binds to the presented antigen, they proliferate and give rise to the GC cell reaction. Second, antigen in association with cytokines released by T cells can rapidly induce differentiation of MZ B cells into plasma cells, which in turn synthesize and release antigen-specific Ig. MALT lymphomas (extranodal marginal zone lymphomas) and monocytoid B-cell NHLs (nodal marginal cell lymphomas) are included within the WHO terminology as marginal zone

lymphomas. Both types share the presence of positive surface immunoglobulin CD19, CD20, and CD22 and are negative for CD5 and CD23. Monocytoid B-cell lymphoma is the nodal form of marginal zone lymphoma. These patients do well, with rates of disease-free and overall survival similar to the other low-grade lymphomas, as shown in studies conducted by the Southwest Oncology Group (SWOG). [3]

Extranodal Marginal Zone Lymphoma

In the WHO classification, the term *extranodal marginal zone lymphoma* is restricted to tumors consisting of small elements provided with centrocyte-like or monocytoid morphology and associated or not with plasmacytoid differentiation, which resemble normal MALT MZ cells and share with them phenotypic and molecular characteristics, including the IRTA-1 gene expression. In the original description, these neoplasms were called MALT lymphomas.

MALT lymphomas typically arise in the mucosal lymphoid tissue or glandular epithelium, including stomach, salivary glands, lungs, or thyroid, with gastrointestinal tract involvement being the most common presentation. There is a clear association with autoimmune diseases such as Sjogren syndrome and Hashimoto thyroiditis. Molecular analysis demonstrates that extranodal MZLs are characterized by the occurrence of different chromosomal aberrations, t(11;18), t(1;14), and t(14;18), which influence invasive potential and possibly the response to therapy. t(11;18)(q21;q21) is detected in 30% to 35% of gastric extranodal MALT lymphomas producing the fusion gene *API2-MALT1*, leading to the overexpression of the *API2* gene, which inhibits apoptosis via the caspase system. Presence of the t(11;18) is associated with antibiotic

resistance, a higher potential of local infiltration and metastasis and progression to a more aggressive tumor. This translocation is found even more frequently in the lung than in the stomach and is found also in approximately one-half of the rare examples of gastric *Helicobacter pylori* (HP)-negative MZL, further supporting the concept that tumors carrying t(11;18) do not need HP stimulation for their growth and maintenance. The t(1;14)(p22;q32) is exceedingly rare and causes transfer of the *BCL10* gene close to the Ig enhancer on chromosome 14. The role of the t(14;18)(q32;q21) in MZL has been the subject of much debate in the literature, with confusion with the translocation found in FL. Although the t(14;18) of MZLs does not affect *BCL2*, it affects *MALT1* by possibly following the same pathogenetic pathway as the t(1;14). Bacterial infection with the gramnegative rod *H pylori* is associated with 92% of gastric MALT lymphomas.

Large B-cell lymphoma can arise at an anatomic site containing MALT, which has been named "high-grade MZ/MALT lymphoma," a term not included in the WHO classification. There is no evidence that large B-cell lymphoma occurring de novo at a MALT site is derived from MZ cells; and the clonal relationship between an MZL and a large B-cell neoplasm simultaneously detected in the same organ should be proven molecularly, as the latter can represent the blastic phase of the former, but might also develop as a second unrelated neoplasm. [3]

Nodal Marginal Zone Lymphoma

In the WHO classification, nodal marginal zone lymphoma (NMZL) is defined as a primary nodal B-cell neoplasm that morphologically resembles lymph nodes involved by MZ lymphoma of the extranodal or splenic type, but

without evidence of extranodal or splenic disease. This suggests that the terms *extranodal*, *nodal*, and *splenic MZL* refer to different clinical presentations of the same disease.

Nodal marginal zone lymphoma is clinically more aggressive than the extranodal and splenic forms and has a higher incidence of advanced-stage disease and lower 5-year overall and disease-free survival. In addition, 10% to 20% of cases transform into a DLBCL. Most cases display a distinct "monocytoid" appearance, and the tumor was originally termed *monocytoid B-cell lymphoma*. Molecular studies strengthen the concept of significant differences among the three types of MZL. The t(11;18) does not occur in nodal MZL. Analysis of IgVH gene demonstrates that some nodal MZLs carry somatic mutations whereas others do not, suggesting derivation from post-GC and virgin B cells, respectively. Among mutated cases, usage of specific IgVH gene segments seems to occur frequently and to discriminate between HCV-positive and -negative patients. None of the translocations characteristically recorded in splenic MZL, including del(7q), del(13q14), and del(10)(q22,q24), has been detected in nodal MZL. [3]

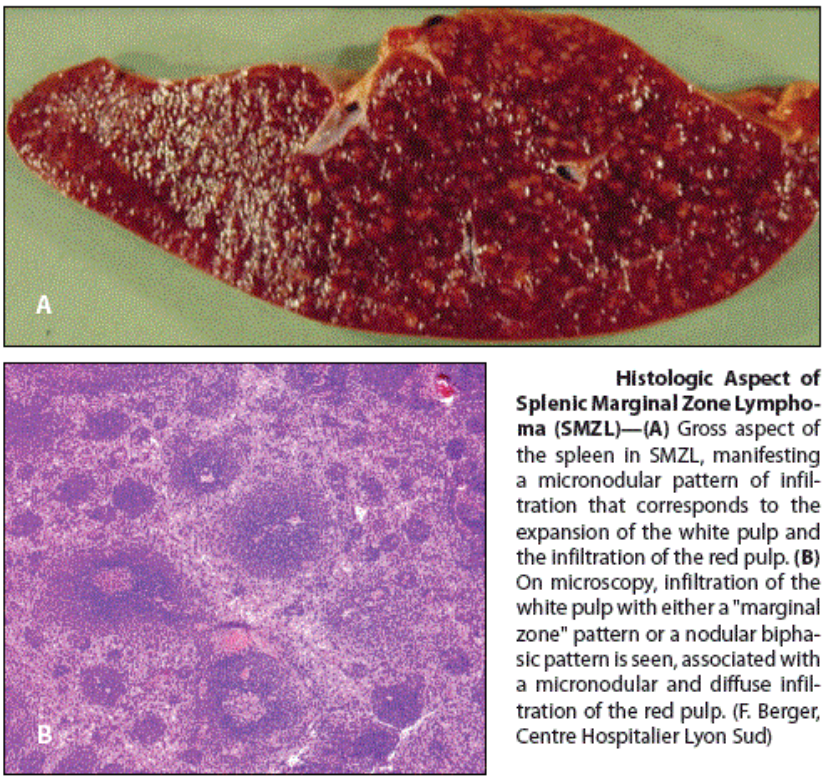
Splenic Marginal Zone Lymphoma

Splenic marginal zone lymphoma is generally characterized by splenomegaly and leukemic spread, even if at times cases with disseminated disease or exclusively leukemic presentation have been reported. In approximately half of the cases, circulating neoplastic cells display cytoplasmic villous projections, which justify the term *splenic lymphoma with villous lymphocytes*. Infiltration of the marrow occurs in most if not all patient S. Molecular studies shows that splenic MZL is a heterogeneous tumor with chromosomal abnormalities including del(7q), del(13q14), and del(10)(q22,q24). Splenic Marginal Zone Lymphoma (SMZL) is a rare B-cell indolent lymphopoliferative disorder (less than 2% of all Non Hodgkin's lymphomas - NHL) that characteristically affects elderly or middle age patients, with median survival longer than 10 years [1-4]. Malignant cells present features of mature activated B-lymphocytes, with expression of CD19, CD20, CD22, CD79b, FMC7, and with light chain restriction. In the majority of cases, neoplastic lymphocytes are negative for CD5, CD23, CD10, and CD103. Bone marrow infiltration pattern is typically intrasinusoidal, but it can become nodular during disease progression or after splenectomy [5,6].

SMZL characteristically presents massive splenomegaly, abdominal discomfort, lymphocytosis and cytopenias, often related to hypersplenism. Lymphonodes and/or organ involvement are infrequent at the diagnosis, but they may develop with progression of disease. B symptoms are present in 25% up to 60% of cases, and autoimmune phenomena are not uncommon (15-20% of patients). Serum paraproteinemia, usually less than 20 g/L, is observed in about 10-25% of patients. Association with HCV infection has been registered

more frequently in South Europe countries, supporting a role of HCV in lymphoma genesis. No specific prognostic factors have been still established for SMZL: high tumour mass, hemoglobin level less than 12 g/dl, increased LDH, albumin less than 3.5 g/dL, and increased beta2 microglobulin at diagnosis have been described as adverse prognostic factors [1,4,7,8].

In particular, the Intergruppo Italiano Linfomi (ILL) defined a prognostic score system based on LDH, albumin and haemoglobin levels identifying 3 different risk groups: low, intermediate and high [8].



Histologic Aspect of Splenic Marginal Zone Lymphoma (SMZL)—(A) Gross aspect of the spleen in SMZL, manifesting a micronodular pattern of infiltration that corresponds to the expansion of the white pulp and the infiltration of the red pulp. **(B)** On microscopy, infiltration of the white pulp with either a "marginal zone" pattern or a nodular biphasic pattern is seen, associated with a micronodular and diffuse infiltration of the red pulp. (F. Berger, Centre Hospitalier Lyon Sud)

The overall prognosis of SMZL is quite good in most patients. The percentage of patients surviving 5 years from diagnosis is 65 to 75%, often even in the absence of treatment or of a complete response to therapy. There is no clear advantage in a precocious treatment, that is considered indicated only when patients develop significant signs and/or symptoms (severe cytopenias, symptomatic splenomegaly, recurrent infections, systemic symptoms). A

"watch and wait" policy is also reasonable for asymptomatic patients who have moderate cytopenias and no-bulky splenomegaly.

Two-thirds of patients do not have symptoms at diagnosis, and up to one-third of those may never require anti-lymphoma treatment [1,4].

Patients presenting with symptomatic (usually painful) splenomegaly are most often treated by splenectomy. This approach often results in a remission lasting several years. If patients have splenomegaly and are HCV-positive, treatment with the anti-hepatitis drugs, such as alpha-interferon and ribavirin may be considered and offers good rate of responses [9,10].

Patients with advanced disease are candidates for more aggressive therapy; purine analogs, such as fludarabine and cladribine, seem to produce higher response rates, although no standard regimen exists [11,12,13]. *The therapeutic impact of Cladribrine (2-chlorodeoxyadenosine-2-CdA) is not yet defined, with different response rates, up to 80%* [14,15,16].

Moreover, Rituximab showed significant activity in SMZL. Treatment with rituximab in symptomatic patients seems to be superior to splenectomy and often leads to normalizing of blood counts and disappearance of splenomegaly. Rituximab is synergistic with chemotherapy and it is often added to whichever regimen is chosen [17,18,19,20].

Table 1. Immunophenotypic markers in different types of marginal zone lymphoma (MZL).

	Splenic marginal zone lymphoma (SMZL)	Nodal marginal zone lymphoma (NMZL)	Mucosa-associated lymphoid tissue (MALT) lymphoma
Most common Markers			
IgG	+	+	+
IgD	+	- (MALT type NMZL) + (Splenic type NMZL)	-
BCL-2	+	+	+
CD19	+	+	+
CD20	+	+	+
CD22	+	+	+
CD43	-	+ in 50% cases	variable
CD79a	+	+	+
FMC7	+	+	+
CD103	-	-	-
CD5	- Helps differentiate it from chronic lymphocytic leukemia (CLL)/mantle cell (can be positive in rare cases)	-	- (can be positive in rare cases)
CD10	- Helps differentiation from follicular	-	-
CD23	- Helps differentiation from CLL (can be positive in rare cases)	-	- (+ in rare cases)
Cyclin D1	- Helps differentiation from Mantle Cell		-

Treatment of indolent lymphomas

For most cases of indolent lymphoma, the goal of therapy has been to maintain the best quality of life and to treat only when patients develop symptoms. Any alteration to this approach requires demonstration of improved survival with early institution of therapy, or identification of criteria that define patients sufficiently high-risk to merit early therapy. There are many available therapies and no consensus on an optimal first-line or relapse treatment. Despite a paucity of data demonstrating any benefit for early therapy, patients are being treated earlier in their disease course. There is no clear cut treatment pathway for patients with indolent lymphomas and little or no data regarding the optimal sequencing of treatment approaches in these diseases. In the absence of such data, treatment choices remain empiric and should always involve discussion

regarding patient choice and goal of therapy. Decisions concerning therapy are likely to become even more complicated because many novel agents are currently being investigated in preclinical and clinical studies, particularly, novel monoclonal antibodies and agents that alter the antiapoptotic pathways. Enrollment of patients on properly conducted clinical trials should be encouraged until we have a clear-cut established treatment approach that leads to cure for the majority of patients.

Options for treatment of low-grade lymphomas include a watch and wait approach, single-agent chemotherapy, or monoclonal antibody therapy with rituximab, combination chemoimmunotherapy, and the use of autologous or allogeneic hematopoietic cell transplantation (HCT). Patients remaining on an expectant course should be followed every 3 months with history, physical examination, and blood counts, including lactate dehydrogenase. Special attention should be paid to any change in symptoms that might be suggestive of transformation, which should be an indication for repeat biopsy to examine for histologic evidence to confirm transformation. The role of routine repeat scanning remains unclear.

Because there is no clearly defined treatment algorithm for most patients with indolent lymphomas, eligible patients should be included whenever possible in clinical trials. This ensures delivery of optimal care and helps inform design of subsequent trials, hopefully leading to cure. [3]

When to institute therapy

With the exception of patients enrolled in clinical trials assessing the impact of early therapy, expectant management is the treatment of choice for asymptomatic patients with low bulk disease until clear indications for

initiation of treatment are seen. This approach is based on the demonstration of no survival advantage for institution of immediate compared with deferred treatment until time of progression. Three randomized trials, performed in the pre-rituximab era, confirmed no survival benefit for early therapy. In the National Cancer Institute study in 104 newly diagnosed patients with FL, deferred treatment was compared with immediate treatment with ProMACE-MOPP followed by total nodal irradiation. An updated analysis of the data is long overdue, but there was no difference in overall survival (OS) between the two arms at the time of the last analysis. The Groupe pour l'Etude de Lymphome Folliculaire (GELF) used defined criteria for patients in whom immediate therapy was not felt to be indicated and randomized 193 patients to deferred treatment or to receive prednimustine 200 mg/ m2/day for 5 days per month for 18 months or interferon-alpha (IFN-alfa) 5 MU/day for 3 months, then 5 MU three times per week for 15 months. The median OS time was not reached and was the same in all three arms of the study. The British National Lymphoma Investigation compared treatment in 309 patients with asymptomatic advanced-stage, indolent lymphoma in whom 158 patients were randomized to receive immediate therapy with oral chlorambucil 10 mg per day continuously and 151 patients randomized to deferred treatment until disease progression. In both arms, local radiotherapy to symptomatic nodes was allowed. There was no difference in OS or cause-specific survival between the two groups with 16 years' median follow-up. A meta-analysis of more than 2000 patients with early-stage CLL/SLL showed no difference in survival between early versus deferred therapy using alkylating agents.

A major clinical trial question is whether identification of clinical or molecular risk factors can identify which patients are candidates for early therapy. A survival predictor score has also been developed from gene expression profiling studies. The results from this study suggest that the molecular determinants of biological heterogeneity are already present in the diagnostic lymph node biopsies rather than by the later acquisition of secondary genetic changes. A major component of the gene expression prognostic signature is related to immune cells in the tumor microenvironment. Future guidelines for treatment will likely be based on clinical staging systems, genetic profiles, and immune response signatures, but these factors do not yet help us to decide who should receive immediate therapy. [3]

From available data, there is little to suggest that we should change our practice of "watch and wait" for asymptomatic low bulk patient, but data demonstrate that this practice is becoming much less common in the USA. The National Lymphocare Study is a prospective observational study designed to assess presentation, prognosis, treatment, and clinical outcomes in newly diagnosed FL. The treating physician determines management according to clinical judgment with no prescribed treatment regimen and data regarding histology, stage, therapy, response, relapse, and death are recorded. Among 1493 patients enrolled at 237 centers, 26% of initially observed patients had switched to active therapy after a median of 2.8 months on observation since diagnosis, and by the first follow-up visit only 19% of patients continued on watch and wait at 6 months. This observation is in stark contrast to the data from the British National Lymphoma Investigation (BLNI) study demonstrating that (censored for nonlymphoma death) 19% of patients and 40% for those older than 70

years who were randomized to expectant management still did not require therapy at 10 years. [3]

Treatment approaches

Treatment is indicated in patients with symptomatic disease, bulky lymphadenopathy, and/or splenomegaly; risk of local compressive disease; marrow compromise; or rapid disease progression. Once indicated, numerous treatment approaches are available. The concept that the approach can be to "do nothing" or discuss an approach with considerable morbidity and mortality such as hematopoietic cell transplantation is a confusing one for the newly diagnosed patient (as well as for the physician), and considerable consultation time is required to review available treatment approaches. Staging of response in indolent lymphomas is by the revised response criteria. Depending on the treatment approach used, restaging after two to three cycles of therapy can be useful to ensure responsiveness with full restaging after completion of therapy. Whereas curative approaches are being sought in indolent lymphomas, the failure to achieve complete remission (CR) does not have the same implication in indolent lymphomas as in aggressive lymphomas, and a PR may be a sufficient response to therapy to alleviate symptoms.

Optimal first-line treatment is enrollment in randomized clinical trials. In the National Lymphocare Study, academic sites are more likely than community sites to treat patients on clinical trials (12% vs 4%), but it is lamentable that such a small proportion of these patients are enrolled in clinical trials. For patients who are not eligible for or who refuse entry into clinical trials, there are data demonstrating higher response rates and longer duration of responses, and perhaps improved survival with chemoimmunotherapy. Many investigators

favor alkylator- over fludarabine-based regimens for FL, based on concerns regarding the ability to obtain stem cells for later use for autologous HCT in fludarabine-treated patients. It is suggested that more aggressive first-line therapy should be offered to patients who progress within 1 year of presentation, because these patients have a worse outcome. Elderly patients or those with poor performance status remain candidates for single-agent chlorambucil. Single-agent monoclonal antibody therapy is appropriate for patients who chose to avoid chemotherapy and is a reasonable treatment choice based on the results of clinical trials of prolonged or maintenance therapy with rituximab. Although data suggest a survival advantage with the use of IFN-alfa in combination with chemotherapy, this is associated with a significant side effect profile and this agent is rarely used in the USA. Optimal results are seen when radioimmunoconjugates are used earlier in the disease course. There is no indication for the use of high-dose therapy and HCT in first remission in FL except in the context of a properly conducted clinical trial.

Data from the National Lymphocare study demonstrate that chemoimmunotherapy is now the treatment of choice of physicians in the USA. No randomized trials demonstrate a benefit for the addition of anthracyclines, but cyclophosphamide, adriamycin, vincristine, prednisone, and rituximab (CHOP-R) is heavily favored over cyclophosphamide, vincristine prednisone, and rituximab (CVP-R) or fludarabine-based regimens. Choice to initiate therapy was associated with FLIPI, stage, and grade but FLIPI was not associated with the decision to utilize a specific treatment approach. Significant regional and center differences were observed, strongly suggesting that physician preference is the predominant factor that drives initial therapy. For

example, initial “watch and wait” was used in 31% in the Northeast, but in 13% in the Southeast, whereas fludarabine-based chemoimmunotherapy was used in 18% of patients in the Southwest and only 3% in the Northeast. [3]

Alkylating Agents

The alkylating agents chlorambucil and cyclophosphamide with or without prednisone and CVP or CHOP, and other alkylator-based combination chemotherapy regimens, have been the standard of therapy for decades. Single-agent alkylators at different doses and schedules produce overall response (OR) rates of 50% to 75% in FL. Comparable response rates but higher complete remission rates with longer progression-free survival (PFS) are seen with CVP compared to chlorambucil, but there is no survival advantage. The addition of anthracyclines has not improved the response rate or duration of the response, but its use may be associated with a lower risk of histologic transformation. This finding has to be confirmed, particularly in the era of chemoimmunotherapy.

Purine Analogues

The purine analogues have been studied extensively in various types of indolent lymphoma. Fludarabine monotherapy produces response rates of 65% to 84%, with 37% to 47% CR in previously untreated FL patients. In a randomized trial of 381 previously untreated indolent lymphoma patients, CR rates were higher with fludarabine than CVP. Fludarabine combinations result in increased response rates, with 89% CR rate in an Eastern Cooperative Oncology Group trial combining fludarabine and cyclophosphamide, whereas fludarabine and mitoxantrone produced a 91% overall response rate, 43% CR, and 63% 2-year disease-free survival. A higher CR rate was seen with

fludarabine and mitoxantrone (68%) compared to CHOP (42%) in a randomized trial. The use of alkylator-based regimens or purine analog-based regimens appears to vary geographically, suggesting personal preference for the use of regimens in which the clinician has experience, rather than alterations of practice based on the results of the published studies. In CLL/SLL, fludarabine is associated with a higher response rate and longer duration of response than chlorambucil, but no OS advantage. The use of fludarabine in combination with cyclophosphamide is associated with a higher response rate and longer duration of response compared with fludarabine alone in randomized trials. The highest response rates have been with fludarabine, cyclophosphamide, and rituximab. [3]

Biologic Therapy

IFN-alfa is approved by the Food and Drug Administration (FDA) for the treatment of advanced-stage FL in combination with anthracycline- based chemotherapy, based on improved survival in a clinical trials and meta-analysis of phase III trial data. IFN-alfa has been widely used in Europe but not in the USA, where it is felt that its toxicity profile outweighs any potential benefit. In the SWOG study, 571 patients with stage III and IV indolent lymphoma were treated with ProMACE-MOPP, and 279 responding patients were randomized to 24 months of observation versus treatment with IFN-alfa. No statistically significant difference in PFS or OS was observed between observation and IFN-alfa groups at 4 years. [3]

Monoclonal Antibody Therapy

Monoclonal antibodies are the most exciting agents to emerge in the treatment of indolent lymphomas, and recent data suggest their use may finally be

leading to improvement in patient survival. The most widely used monoclonal antibody is rituximab, a chimeric unconjugated antibody against the CD20 antigen licensed by the FDA and the European Agency for the Evaluation of Medicinal Products for treatment of patients with relapsed or refractory, CD20-positive low-grade FL; for the first-line treatment of CD20- positive FL in combination with CVP chemotherapy; and for the treatment of CD20-positive low-grade NHL in patients with stable disease or who achieve a PR or CR following first-line treatment with CVP chemotherapy.

Following phase I studies, rituximab at a dose of 375 mg/m2 weekly for 4 weeks was selected for the pivotal phase II trial and this remains the standard dose. In relapsed indolent lymphoma patients, OR was 48% and 60% in FL. Median PFS for responders was 13 months. Factors associated with lower response rates include chemoresistant disease, bulky disease, and treatment late in the disease course. The incidence of OR was 73% in previously untreated patients with low bulk disease, and some of these patients have needed no further treatment and have no evidence of polymerase chain reaction-detectable minimal residual disease after 7 years. Extended use with 8 weeks instead of four is associated with improvement in OR and duration of response. Comparable or even longer durations of response have been observed with retreatment.

A number of trials in front-line and in relapsed/refractory patients have investigated the potential benefits of extended or maintenance rituximab treatment and all demonstrated prolonged time to progression in patients receiving maintenance rituximab. The results from the E1496 randomized trial from the Eastern Cooperative Oncology Group and the Cancer and Leukemia

Group B comparing CVP alone to CVP followed by rituximab in patients with advanced-stage FL demonstrated that addition of rituximab maintenance significantly improved OS and led to FDA approval for rituximab therapy in patients responding to CVP chemotherapy. A problem with interpretation of the role of maintenance therapy or in recommending a specific regimen is that there is no standard schedule and trials have been performed in rituximab-naïve patients as well as in patients treated with previous rituximab monotherapy or combination chemoimmunotherapy.

Chemoimmunotherapy

In a phase II study, 40 patients with indolent lymphoma were treated with six infusions of rituximab (375 mg/m2 per dose) in combination with six doses of CHOP chemotherapy (R-CHOP). Overall response was 95%, with a CR of 55% and OR of 45% in patients with bulky disease. In a phase II study of 40 patients with indolent lymphomas, rituximab in combination with fludarabine produced OR of 90% and CR of 80%, with similar response rates in treatmentnaïve and previously treated patients.

A number of randomized trials show a benefit for the use of rituximab with chemotherapy compared to chemotherapy alone. Each study showed an improvement in time to treatment failure. More recent follow-up data suggest improved OS in patients treated with chemoimmunotherapy compared to chemotherapy alone. A meta-analysis of these trials demonstrates that OS, OR, and disease control are significantly better in those on chemoimmunotherapy compared to chemotherapy for FL and mantle cell lymphoma. Data from the German Low-Grade Study Group suggest that it is the addition of rituximab that has led to the recent improvement in survival of patients with FL. A recent

independently assessed analysis of the clinical benefits provided by rituximab in relation to cost concluded that it is highly cost-effective.

Conjugated Radiolabeled Monoclonal Antibody Therapy

Binding a radioisotope to a monoclonal antibody (radioimmunoconjugate) might be expected to improve efficacy over antibody therapy alone. Tositumomab joins 131I to the anti-B1 antibody and has been studied extensively in the treatment of heavily pretreated and untreated lymphomas and for retreatment of indolent lymphomas. Best responses are seen in previously untreated FL patients with a 95% OR and 75% CR. Eighty percent of assessable patients achieved eradication of polymerase chain reaction-detectable minimal residual disease after a single treatment course with tositumomab. Median PFS was 6.1 years, with 40 patients remaining in remission for 4.3 to 7.7 years and no cases of myelodysplastic syndrome observed. A SWOG study investigated chemoimmunotherapy with six cycles of CHOP chemotherapy followed 4 to 8 weeks later by tositumomab in 90 patients with previously untreated, advancedstage FL. The OR was 91%, including 69% CR and at median follow-up time of 5.1 years, the estimated 5-year OS was 87% and PFS 67%. These results were significantly better than results of therapy with CHOP alone on previous SWOG protocols. Ibritumomab Tiuxetan is a 90Y-labeled anti-CD20 antibody and produced an OR of 74% and CR of 15% in 57 FL patients refractory to rituximab. Toxicity is primarily hematologic, with nadir counts occurring at 7 to 9 weeks and lasting approximately 1 to 4 weeks. The risk of hematologic toxicity increased with dose delivered and with degree of baseline marrow involvement. An acceptable safety profile was observed in relapsed patients with less than 25%

lymphoma marrow involvement, adequate marrow reserve, platelets greater than 100,000 cells/microL, and neutrophils greater than 1500 cells/microL.

High-Dose Therapy as Consolidation of First Remission

The role of high-dose therapy and autologous HCT in FL patients during first remission was explored in phase II trials, and in three phase III randomized trials. The German Low-Grade Study Group trial recruited 307 previously untreated patients up to 60 years of age. Patients who responded after induction chemotherapy with 2 cycles of CHOP or mitoxantrone–chlorambucil–prednisone were randomized to autologous HCT or IFN-alfa maintenance. Among 240 evaluable patients, the 5-year PFS was 64.7% for autologous HCT and 33.3% in the IFN-alfa arm (P = .0001). Acute toxicity was higher in the autologous HCT group, but early mortality was below 2.5% in both study arms. Longer follow-up is necessary to determine the effect of autologous HCT on OS. In the Groupe Ouest Est des Leucemies Aigues et des Maladies du Sang study, 172 newly diagnosed advanced FL patients were randomized either to cyclophosphamide, doxorubicin, teniposide, prednisone (CHVP) and IFN-alfa or to high-dose therapy followed by purged autologous HCT.105 Patients treated with high-dose therapy had a higher response rate than patients who received chemotherapy and IFN-alfa (81% vs 69%, P = .045) and a longer median PFS (not reached versus 45 months), but this did not translate into a better OS because of an excess of secondary malignancies after transplantation. A subgroup of patients with a significantly higher event-free survival rate could be identified using the FLIPI. The GELF-94 study enrolled 401 previously untreated advanced-stage FL patients who were randomized to receive CHVP plus IFN-alfa compared with four courses of CHOP followed by

HDT with total body irradiation and autologous HCT. Overall response rates were similar in both groups (79% and 78%, respectively), and 87% of eligible patients underwent autologous HCT. Intent-to-treat analysis after a median follow-up of 7.5 years showed no difference between the two arms for OS (*P*=.53) or PFS (*P* =.11). Long-term follow-up demonstrated no statistically significant benefit in favor of first-line autologous HCT in patients with FL. In view of these results, autologous HCT should be used in first remission only in the setting of clinical trials.

Treatment of relapsed indolent lymphoma

The treatment options after relapse remain the same as for first-line therapy, and relapsed patients should ideally be treated in clinical trials. Relapsed asymptomatic disease is not necessarily an indication for treatment and patients can again be managed expectantly. A number of factors must be taken into account in planning therapy and it is not possible to define treatment at relapse without considering the goal of therapy (palliative vs potentially curative) performance status, previous therapy, response, and duration of response. Single agent rituximab is approved for relapsed lymphoma and is widely used in this setting. A multicenter randomized trial in relapsed patients has demonstrated a survival advantage for chemoimmunotherapy with CHOP-R or CHOP followed by R compared to CHOP alone, and a further benefit for rituximab maintenance therapy. For younger patients who are suitable candidates for autologous HCT or reduced-intensity conditioning (RIC) allogeneic transplantation, referral to a transplant center should be considered early to discuss the potential role and timing of transplantation. Best results are seen when transplantation is considered early in the course of disease before

patients become chemorefractive. Hematopoietic cell transplant approaches must be considered in the context of the improving results that are being seen with salvage therapy alone. The results of autologous HCT have been disappointing for CLL/SLL; however, RIC allogeneic transplants appear promising in selected patients with this disease.

The Role of Transplant in Relapsed Indolent Lymphomas

Unlike aggressive lymphomas, the use of high-dose chemotherapy with autologous HCT in the treatment of indolent lymphomas has not yet been fully established. The rationale for considering transplantation is that the disease is incurable using standard approaches, and promising results have been observed in a number of phase II studies. Detection of minimal residual disease has been a useful surrogate marker for tracking long-term PFS in patients examining the autologous stem cells or serial samples after transplantation. A major concern relates to the risk of secondary myelodysplasia/acute myeloid leukemia. The European Bone Marrow Transplant Registry- sponsored CUP study (conventional chemotherapy, unpurged, purged autograft) is the only prospective randomized trial to assess the role of autologous HCT in patients with relapsed FL. The results of the study suggest a PFS and OS advantage of autologous HCT over conventional chemotherapy, with a 4-year OS of 46% for the chemotherapy arm, versus 71% for the unpurged and 77% for the purged autologous HCT arms. The study was closed early because of slow accrual with 140 of the planned 250 patients accrued and only 89 randomized. In CLL/SLL, the use of autologous HCT was not associated with improved outcome in patients transplanted in first remission compared to those transplanted later in their disease course.

Allogeneic Hematopoietic Cell Transplant

There is a trend toward increasing use of allogeneic HCT in the management of indolent lymphomas. In a report of the International Bone Marrow Transplant Registry, results after HCT are described for 904 patients with FL. Among these patients, 176 patients underwent allogeneic HCT, and 131 patients underwent autologous HCT using a purged inoculum and 597 using an unpurged autologous inoculum. The treatment-related mortality (TRM) in these three groups was 30%, 14%, and 8%, respectively, disease recurrence in 21%, 43%, and 58% and 5-year OS was 51%, 62%, and 55%, respectively. The use of total body irradiation-containing regimens was associated with increased TRM but decreased risk of relapse. The use of allogeneic HCT was associated with increased TRM but significantly lower risk of disease recurrence in keeping with a graftversus- lymphoma effect in this disease. It should be noted that the majority of allogeneic transplant recipients reported in these studies received a fully myeloablative regimen. Long-term PFS has been observed after allogeneic SCT even in patients with refractory FL. In 29 FL patients, 11 of whom had refractory disease, the nonrelapse mortality was 24% and there was a 23% incidence of relapse. The 5-year OS was 58%, with 53% event-free survival. Patients who have relapsed after previous autologous HCT have a very poor prognosis. The outcome following myeloablative allogeneic HCT of 114 such patients has been reported from the International Bone Marrow Transplant Registry. The TRM was 22% and the probability of disease progression was 52% at 3 years. The use of total body irradiation conditioning regimens and achievement of CR at the time of allogeneic HCT were associated with improved outcome. The use of RIC regimens appears to be

associated with improved outcome. In 20 such patients, there was only one TRM from fungal infection and the 3-year progression-free survival was an excellent 95%. The out-come following RIC transplant regimen incorporating alemtuzumab immunosuppressive therapy has been reported for 81 patients with lymphoma, including 41 with low grade, 37 with high/intermediate grade, and 10 patients with MCL, 31 of whom had relapsed following previous autologous HCT. Patients received a conditioning regimen consisting of alemtuzumab, fludarabine, and melphalan, and received short-course cyclosporine as GVHD prophylaxis. The use of this conditioning regimen was associated with a low incidence of GVHD and TRM was decreased in patients with low-grade compared to higher-grade histology. The 3-year progression-free survival was 65% for patients with low-grade lymphoma, 50% for patients with MCL, and 34% for high-grade lymphoma ($P = .002$). Donor lymphocyte infusion was given to 36 patients, 21 for relapsed or persistent disease and 15 for persistence of mixed chimerism. Investigators hypothesize that the use of donor lymphocyte infusion to treat relapse after allogeneic HCT will stimulate an effective graftversus- lymphoma response. In one series, seven patients with FL and SLL who had relapsed after prior allogeneic HCT received donor lymphocyte infusion. Six patients responded and four experienced CRs, which have been maintained for 43 to 89 months. The effectiveness of donor lymphocyte infusion to treat relapse after allogeneic HCT provides very strong evidence for a graft-versuslymphoma effect that can be exploited in indolent lymphomas. [3]

Special consideration for treatment of SMZL

There are still contrasting opinion about the optimal therapeutic approach in this disease. Most studies have suggested better outcomes for those patients who underwent splenectomy, suggesting that splenectomy may be the first-line treatment choice, but this can be delayed until the occurrence of symptoms or cytopenia and seems to be sufficient for correcting cytopenic manifestations, improving quality of life and increasing survival (with median values of between 9 and 13 years). The utility of alternative approaches, including chemotherapy, radiotherapy, or immunotherapy, is the subject of prospective clinical trials. Adverse prognostic predictors include hemolytic anemia, immune thrombocytopenia, M-component in the serum, elevated beta2-microglobulin level, leukocyte count >20,000/microL, lymphocytes >9000/microL, and p53 overexpression by neoplastic cells. Progression to DLBCL has been recorded rarely. [3]

Novel agents of indolent lymphoma

Overall, iNHLs are highly responsive to standard chemotherapy regimens, but still remain incurable, showing a relentless progressive-relapsing course. In the pre-rituximab era median survival for advanced iNHL was around 8-10 years and after relapse it became around 4-5 years. In the last decade, however, the advent of rituximab-based immunochemotherapy not only increased overall response rate (ORR) and complete response (CR) rate, but prolonged progression-free survival (PFS) and overall survival (OS). Furthermore, the introduction of the radio-immuno-conjugates 90-yttrium labelled ibritumomab tiuxetan and 131-iodine labelled tositumomab has shown promising results in terms of efficacy and safety in either front-line, consolidation or

relapsed/refractory settings, including in some cases long-term remissions. In addition, the increasing understanding of molecular mechanisms involved in the pathogenesis of iNHL has opened the door to the discovery and development of several new targeted therapies that in the near future could challenge the current scenario. [4]

Chemotherapeutic agents

BENDAMUSTINE: bendamustine is an 'old' alkylating agent that has been 'rediscovered' recently and has undergone extensive clinical development. It was first synthesized in 1963 by Ozegowski and it has been used in Germany since 1971 for the treatment of several haematologic malignancies. The chemical structure of bendamustine consists of a nitrogen mustard linked to a benzimidazole ring, which is thought to confer purine-analogue properties, and a butyric acid side chain. Experimental evidence of its unique mechanism of action has demonstrated: (1) a potent ability to induce sustained double strand breaks; (2) the induction of unique DNA repair; (3) the induction of apoptosis via extrinsic and intrinsic pathways (p53, NOXA, caspases cascade); (4) the deregulation of the cell cycle through the inhibition of mitotic checkpoints; and (5) the induction of apoptosis-independent forms of cell death ('mitotic catastrophe'). As a result, bendamustine displays significant mechanistic differences from other alkylating agents, exhibiting greater stability and slower repair of DNA damage. In preclinical models bendamustine demonstrated significant synergism with rituximab.

Beginning in the late 90s, many German groups have performed an increasing number of pilot trials evaluating bentamustine in NHL. These studies showed a

remarkable activity and a very favourable side-effects profile, characterized primarily by moderate haematological toxicity, mild nausea and a quite inconsistent rate of alopecia. Two large US phase II multicentre studies investigated bendamustine monotherapy in patients with rituximab refractory in iNHL. In the first, bendamustine showed a favourable side-effect profile with good clinical activity: grade 3-4 toxicities were prevalently hematological, including reversible neutropenia (54%) and thrombocytopenia (25%); the ORR was 77% (CR/CRu 34%), with a median duration of response (DOR) of 9 months.The second study showed similar results (ORR 75%, CR/CRu 17%; median PFS 9.3 months). Of note, the efficacy of bendamustine was comparable between the different indolent histological sbtypes: the ORR was 74% in FL, 71% in SLL, 86% in MALT-MZL and 78% in NMZL patients. Taken together, these studies demonstrated a promising clinical activity and a good safety profile for bendamustine in patients with rituximab-refractory iNHL. However, the duration of remission was rather short. [4]

To improve these results, the logical next step was to combine bendamustine with rituximab (BR). Two similar trials investigated this combination in relapse/refractory iNHL. In the first study, Rummel and collegues treated 63 patients with FL, iNFL or MCL with the BR regimen. Leukopenia was the most common side-effect (16% grade 3-4); no evidence of cumulative myelosuppression was found. None of the patients suffered from alopecia and no organ toxicity was seen. The response rate was promising (ORR 90%, CR 60%) and, importantly, responses were fairly durable, with median PFS of 24 months. Notably, PFS in FL and PLP patients was even higher (median not

reached). The second trial showed almost identical results, in term of toxicity profile (36% grade 3-4 neutropenia) and efficacy (median PFS of 23 months).

As a consequence of these results in the relapsed/refractory setting, the StiL group performed a phase III randomized study comparing six cycles of BR with six cycles of R-CHOP as first-line therapy in 514 patients with FL, iNFL and MCL. The final results of this study were reported at the ASH meeting in 2000 and updated at the last ASCO meeting. BR was more effective than R-CHOP with a median PFS of 69.5 months *versus* 31.2 months. The advantage in term of PFS was evident in all risk groups and histologic subtypes, with the exception of the small subgroup of MZL. OS was not significantly different between the two treatment regimens. Concerning adverse events, R-Chop was more toxic than BR (grade 3-4 neutropenia 46.5% *versus* 10.7%). In addition, a sub-analysis of this study showed that the BR combination did not impair the collection of stem cells for subsequent transplant, since the mobilization performed at the end of the treatment course allowed a similar rate of success in both arms.

In a similar way, the StiL group performed a phase III study that compared six cycles of BR and fludarabine-rituximab in 208 patients with relapsed/refractory iNHL or MCL. BR showed a superior efficacy, with a better ORR (83.5% *versus* 52.5%), CR (38.5% *versus* 16.2%) and PFS (30 *versus* 11 months), without any difference in terms of toxicity.

Since bentamustine in an alkylating agent, a great deal of attention has been focused in the issue of secondary malignancies. To date, the two StiL studies did not show an increased rate of MDS/AML or solid tumors in the BR arm; however, a longer follow up is still warranted to draw definitive conclusions.

For the future directions, bendamustine is now being evaluated in relapsed/refractory iNHL in conjunction with a great variety of other agents (i.e. IMIDs, proteasome inhibitors, monoclonal antibodies [mAbs]). [4]

Agents that target the cell surface:

Novel mAb anti CD-20

As mentioned previously, the introduction of the chimeric human-mouse mAb anti CD-20 rituximab actually changed the outcome of B-cell NHL and the combination of rituximab and chemotherapy is now the standard treatment inNHL. Nevertheless, a significant number of patients affected by iNHL develop recurrent disease and become refractory to first or subsequent immunochemotherapy treatment. In these cases retreatment with rituximab-based therapy is not considered useful and new agents potentially able to overcome rituximab-resistance mechanisms are recommended. For this reason, several novel mAbs, either directed against CD20 or other antigens, were specifically designed by incorporating structural modifications that are hoped to overcome mechanisms of rituximab resistance. Many of these new mAbs are currently under different preclinical and clinical phases of assessment.

Ofatumumab

Ofatumumab is a fully humanized anti-CD20 mAb. Classified as a type I mAb, ofatumumab displays greater complement-dependent cytotoxicity (CDC) with respect to rituximab [Telling *et al.* 2004]. Ofatumumab binds to a novel epitope of CD20, which encompasses the small extracellular loop and the N-terminal region of the second large extracellular loop. Ofatumumab binds to the CD20 with greater avidity than rituximab and his action is carried out even at lower density of this cell surface antigen.

In the field of iNHL, Ofatumumab has been initially tested in FL. In the first phase I/II study, Hagenbeek and coworkers enrolled 40 patients (37.5% previously treated with rituximab) affected by relapsed or refractory FL. The best response rate across all dose groups was 43%, without direct correlation with ofatumumab dose. Previous rituximab-treated patients had a response rate of 64%. The median time to progression (TTP) was 8.8 months for all patients and 32.6 months for responders, with median DOR of 29.9 months. Ofatumumab was generally well tolerated and toxicity was similar to that of rituximab (reversible grade 3-4 infusional reactions occurring during first infusion). Immunological tests showed that ofatumumab induced a longer lasting depletion of B cells (6-10 months) compared with rituximab. However,infections were infrequent and generally mild (18 grade 1-2 and 2 grade 3).

To assess whether this novel anti CD20 mAb might overcome rituximab resistance, ofatumumab was tested in 116 rituximab-refractory FL patients in a double-blind study evaluating two dose levels. Patients were heavily

pretreated: 65% had chemotherapy-refractory disease and the median number of previous treatments was 4. The subjects who received the higher dose experienced an ORR of 10% (1 CR, 8 PR) while 50% had stable disease (SD). Median DOR was 6 months. The limited activity in this setting suggests it should be combined with chemotherapy (CHOP or bendamustine).

A subsequent phase II study explored the combination of ofatumumab with CHOP in 58 patients with previously untreated FL. In the higher dose group ORR was 100% (CR 38%). The most common grade 3-4 adverse events were leukopenia and neutropenia.

In conclusion, ofatumumab has demonstrated efficacy as a single agent in relapsed/refractory FL patients, with less evident effect in true rituxmab refractory patients. The combination of ofatumumab and CHOP appears highly active in previously untreated FL and opens the way to further studies.

Obinutuzumab (GA-101)

Obinutuzumab (GA-101) is a third-generation, fully humanized, type II anti-CD20 mAb, that was glyco-engineered to display afucosylated Fc region carbohydrates, resulting in enhanced antibody-dependent cell-mediated cytotoxicity (ADCC) activity and superior direct B-cell killing compared with rituximab and other type I mAbs, despite lower complement-dependent cytotoxicity (CDC) activity. GA-101 was investigated as a single agent in two phase I studies. In the first trial, 21 patients with relapsed/refractory B-cell NHL were treated with escalating doses of GA-101. All patients were heavily pretreated; 20 (95%) were previously exposed to rituximab and 9 (43%) were

rituximab-refractory. No dose-limiting toxicity was observed, and side-effects consisted primarily of grade 1-2 infusion- related reactions. An overall response was observed in nine patients (43%), including five CR/CRu. All responding patients belonged to the FL subgroup (ORR 69%, CR 38%). The phase II part of this study compared two different does of GA-101 in 40 patients with relapsed/refractory FL. Better results were obtained in the higher dose group (ORR 60%, CR/CRu 33%; median PFS 11.8 months). These preliminary data indicate that GA-101 monotherapy display encouraging efficacy with higher response observed at higher dose. In the second phase I trial, 22 subjects with B-cell NHL or chronic lymphocytic leukemia (CLL) received four weekly does followed by maintenance therapy. Half of patients were refractory to prior rituximab. Toxicity was similar to the previous trial. After completion of induction, 5 patients obtained a PR (22%) and 12 had SD; 8 patients received maintenance treatment, that lead to improvement of response in 3 patients. In the subset of FL, the best ORR was 40% (4/10 including 1 CR). [4]

Preliminary results of the first head-to-head trial of obinutuzumab against rituximab in relapsed/refractory iNHL patients have been presented at the last ASH meeting in San Diego (2011) and updated at the last EHA meeting in Amsterdam (2012). A total of 175 patients were randomized to recive four weekly infusions of either GA-101 or rituximab. Patients had received a median a median of two prior treatments (prior rituximab in 99). Safety analysis did not reveal any difference between the two treatments. Based on blinded central radiology review, ORR was significantly in favour of GA-101

(44.6% versus 26.7%); however, at the time of analyses (median observation time: 15 months), PFS and OS were not different between the two arms. Longer follow up is probably needed to establish whether the higher response rate of GA-101 with respect to rituximab could be able to translate also in better survival.

Given its encouraging antilymphoma activity, particularly in FL, obinutuzumab has been evaluated also in combination with chemotherapy in relapse/refractory FL setting. In a phase I study (GAUDI), 56 patients were randomized to receive either 6-8 CHOP or 4-6 FC cycles, in combination with GA-101. All patients (28/28) in the G-CHOP arm and 22/28 in the G-FC arm completed the treatment. Grade 3-4 neutropenia was reported in 39% and 50% of patients treated with G-CHOP and G-FC, respectively. ORR at the end of induction was 96.4% in G-CHOP group (39% CR) and 92.9% in G-FC group (50% CR). The response rate with G-CHOP compared favourably with the R-CHOP arm of a similar previous trial (EORTC 20981). In conclusion GA-101 can be combined safely with CHOP, demonstrating a high level of activity compared with historical controls, while G-FC showed worse tolerability.

Following these promising results, obinutuzumab is currently being explored with chemotherapy (CHOP, CVP or bentamustine) as first-line therapy in a randomized phase III study against the current standard of care rituximab chemotherapy in patients eith advanced untreated iNHL.

Antibodies against target other than CD20

CD22 is an antigen widely expressed on normal and malignant B cells and plays a role in B cell receptor (BCR) activation and signal transduction.

Epratuzumab is a humanized anti-CD22 antibody that demonstrated ADCC and direct cytotoxicity in preclinical studies [Leonard *et al.* 2005]. Phase I/II studies showed that epratuzumab is well tolerated, and has significant single-agent clinical activity across various dose levels in relapse/refractory FL, suggesting its combination with rituximab should be explored. In the relapse/refractory setting this combination demonstrated promising results, with an ORR of 54% in FL patients (CR 24%) and a media DOR of 13.4 months. Also in first-line setting this regimen seems to be quite effective, showing an ORR of 84% (CR 33.3%) in 57 FL patients.

Galiximab is a Chimeric human-primate anti-CD80 mAb with single-agent activity and excellent tolerability in previous treated FL. Aphase I/II study evaluating the combination of galiximab and rituximab in patients with relapsed/refractory FL showed an ORR of 66% and a median PFS of 12.1 months. In 61 untreated FL patients, this regimen appeared efficacious (ORR 72%, CR/CRu 47%), particularly in patients with low-risk Follicular Lymphoma International Prognostic Index (FLIPI) score (ORR 92%, CR/CRu 75%, 75% 3-year PFS 75%).

Immunoconjugates

Another mechanism to induce cell killing beyond the direct antibody effect is represented by the use of a toxin conjugated to B-cell mAbs. Differently from naked antibodies, which exert their effects largely from the cell surface, conjugated antibodies often benefit from internalization. Inotuzumab ozigamicin (CMC-544) is a humanized anti-CD22 antibody conjugated to calicheamicin. In a phase I study the main toxicities included

thrombocytopenia, asthenia, nausea, neutropenia and elevated aspartate transaminase (AST). The ORR for all patients was 39%; 68% of patients with FL responded at the maximum tolerated dose. A phase II study evaluating the combination of inotuzumab ozigamicin and rituximab enrolled 110 patients with refractory or recurrent FL or aggressive NHL.In 38 patients with FL, ORR was 87%and median PFS was 23.6 months.

Bispecific antibodies.

Bispecific antibodies are antibodies that target two antigens. Blinatumomab is an anti-CD3/anti-CD19 antibody that engages cytotoxic T cells and malignant B cells, enhancing tumor lysis. Phase I studies have demonstrated tolerability and clinical activity in B-cell NHL. Further studies are now evaluating this promising new treatment in different NHL subtypes.

Agents that target intercellular processes

Proteasome inhibitors

Bortezomib

Bortezomib is a first-in-class drug designed to target the ubiquitin-proteasome complex; i.e. the regulatory pathway that exerts intracellular protein degradation in eukaryotes, resulting in impairment of apoptosis, that is considered one of the most involved pathways in the pathogenesis of iNHL. For this reason bortezomib has been evaluated with great interest in FL and iNFL (especially WM).

Early phase II trials revealed striking variation of efficacy in different histologies: while significant response rates bortezomib were shown in MCL.

The results in other iNHLs were less impressive. The first trial addressing the efficacy of biweekly (biw) single-agent bortezomib at standard dose (1.3 mg/mq) in FL showed dismal results, with a median TTP of 5.1 months. Best response rates were found with higher dose (1.5 mg/mq) in 18 patients with FL, with 50% ORR (22% CR), similar to the MCL group; however, the toxicity of this schedule, mainly in terms of grade 3-4 periferal neuropathy (PN) (8%) and thrombocytopenia (27%) was not negligible and led to frug discontinuation in 33% of subjects. In order to ameliorate toxicity, a weekly (qw) bortezomib schedule (1.8 mg/mq) was studied in 26 iNHL patients; however, the ORR was low (18%)and PFS was not improved (6.7 months). The weekly vs twice-weekly debate was answered by the GELA in a randomized phase II trial comparing to the two schedules of single-agent bortezomib in 87 relapsed/refractory FL patients. At planned interim analysis the weekly dosing arm demonstrated insufficient responses (ORR 23% vs 32%) and twice-weekly schedule was recommended for furthers studies. However, given the unsatisfactory response rates of single-agent bortezomib, the focus moved to the combination of bortezomib with rituximab or immunochemotherapy regimens in the hope of improving efficacy in FL and iNFL.

The combination of bortezomib and rituximab is an attractive area of investigation because of the different mechanism of action and side-effect profiles; moreover, in vitro and in vivo murine studies showed synergistic activity. One of the first studies addressing this combination explored again the weekly versus twice-weekly bortezomib schedule with the association of rituximab in 81 patients with relapsed/refractory FL or MZL. As expected, the

weekly schedule was better tolerated (grade 3-4 PN 5% versus 10%) and given the non inferiority in terms of efficacy, it was chosen for further develpoment. To this purpose, a large international randomized phase III trial (LYM-300) allocated 676 patients with relapsed/refectory FL to receive rituximab either alone or with bortezomib. Median PFS was 12.8 months in the bortezomib-rituximab arm and 11 months in the rituximab only arm; this also coincided with a better ORR (63% versus 49%). However, the clinical benefit did not reach the anticipates prespecified improvement af 33% in PFS and the safety profile revealed higher rates of PN in a combination arm. Another phase II trial (BRIL-06) evaluating rituximaband bortezomib combination in 49 patients with relapsed/refractory iNFL and MCL, showed encourraging results: ORR 53% (CR, 26.5%), 1-year PFS 50% for MZL and 37% for LPL.

Many studies addressing feasibility and efficacy adding bertozemib to an immunochemotherapy regimen in iNHL patients have been performed in recent years. In a randomized phase II trial of the GELA, patients with B-cell NHL were allocated to receive frontline standard R-CHOP with addition of bortezomib either with the biweekly or weekly schedule. A total of 49 patients were enrolled, with a CR/CRu rate of 82%. The ORR appeared higher in the biweekly arm (90% versus 70%) and at the higher doses. However, since grade 3 neurologic toxicity was excessively increased at the higher doses in both arms, the investigators concluded that the biweekly doses of bortezomib in combination with R-CHOP should not exceed 1 mg/mq. A recent phase I trial in untreated iNHL demonstrated, however, that weekly bortezomib, combined with modified R-CHOP with vincristine capped at 1.5 mg, is able to produce

high response rates without substantial increase in PN development (grade 3-4: 11%) up to the maximum tolerated dose of 1.6 mg/mq.

Recently, two phase II trials investigating the combination of bortezomib, bentamustine, and rituximab (VBR) have been completed. In the larger VERTICAL trial, 73 patients with relapsed/refractory FL were enrolled to receive up to five cycles of VBR. The ORR was 88% (53% CR) and the median PFS was 14.9 months. Myelosuppression was the main toxicity (25% and 14% of patients experienced 3-4 neutropenia and thrombocytopenia, respecively), while transient grade 3-4 neuropathy occurred in 11% of patients. Although the primary endpoint of CR >60% was not accomplished, this regimen demonstrated to be effective in this highly pretreated population. The second study (31 patients) showed comparable results: although ORR was encouraging (83% for all subtypes; 93% for FL), the 2-year PFS of 47% did not meet the primary endpoint of 25% improvement with respect to historical results of bendamustine-rituximab only. In conclusion, neither of these trials demonstrated a significant improvement in ORR and PFS with the addition of bortezomib in relapsed/refractory FL and other iNHL.

As preclinical studies suggested that bortezomib, through inhibition of NF-kB, may act as a radio-sensitizer, a phase I study was designed to explore the feasibility of weekly bortezomib combined with radioimmunotherapy (90Y ibritumomab tiuxetan) in relapse/refractory FL: despite the high rate of hematologic toxicities, this regimen resulted safe, well tolerated and effective. Combination regimen including bortezomib seem to be an attractive option in WM. Recently, Treon and colleagues reported efficacy of bortezomib, dexametasone, and rituximab in 23 previously untreatede patients with

symptomatic WM. As a best response, median bone marrow disease involvement declines from 55% to 10%, serum IgM levels declined from 4830 to 1115 mg/dl, and haematocrit increased from 29.8% to 38.2%. The ORR was 96%, and at a median follow up of 22.8 months, 18 out of 23 patients remained free of disease progression. The most common toxicity was PN, which led to discontinuation of treatment in 61% of patients. [4]

Other proteasome inhibitors

Bortezomib is a reversible proteasome inhibitor that exhibits a lower affinity to target-binding sites, resulting in practical limitation because of drug schedule and intensity. Carfilzomib is a second-generation irreversible and selective proteasome inhibitor: recent data showed that carfilzomib is highly active in relapsed/refractory MM patients, especially if bortezomib-naive. Notably this agent is associated with only minimal painful PN, while its dose-limiting toxicity is myelosuppression. Moreover recent data provided evidence of significant preclinical activity of carfilzomib in WM.

mTOR inhibitors

The PI3K/AKT/mTOR signalling pathway is one of the most deregulated in human cancer and it has been extensively studied as a molecular target in NHL therapy. This pathway regulates cell growth and survival in response to growth factor receptor signalling and metabolic status. The activation af AKT through PI3KT leads to the stimulation of the mammalian target of rapamycin (mTOR) that is a serine/threonine kinase that regulates translation of proteins involved in cell growth, protein synthesis and cell cycle progression; mTOR exerts its

action as a part of two complexes, mTORC1 and mTORC2, involved in the translation of oncogenes such as c-MYC or Cyclin D1.

PI3K/AKT/mTOR activation has been described mainly in MCL; however, preclinical studies have also demonstrated activaion of mTOR pathways in indolent lymphomas such as FL and WM. For these reasons, many drugs targeting PI3K/AKT/mTOR pathways, and in particular rapamycin-analogue (rapalog) mTOR inhibitors, are under investigation in iNHL treatment.

Rapamycin is a macrolide antibiotic that exerts its activity on mTORC1 complex causing a conformational change in its activity site. New molecules with improved bioavailability are temsirolimus and everolimus.

Temsirolimus.

Temsirolimus is a water soluble rapalog that is rapidly converted to the parent compound rapamycin after intravenous administration. It has been mainly studies in MCL for which is now a treatment option in relapsed/refractory disease.

Temsirolimus has been evaluated also in other NHL histotypes: Smith and colleagues published a phase II trial in which temsirolimus was used as a single agent in patients with relapsed aggressive and indolent lymphomas. FL patients reached on ORR and a CR rate of 54% and 26%, respectively, and median PFS of 12.7 months; median OS has not yet been reached; CLL/SLL, and other iNHL patients obtained a PR rate of 11% with no complete responders. If we consider the results obtained by Hess and colleagues in MCL (ORR 22%; median PFS 4.8 months), the results in FL seem promising. This was the first study to establish single-agent activity of temsirolimus in patients with B-cell

lymphomas other than MCL, showing that mTOR inhibition is a rational target also in other subtypes of lymphomas.

Everolimus

Everolimus, an orally available ester derivative of rapamycin, has been tested in a phase I study in advanced haematologic malignancies by Yee and colleagues: no dose-limiting toxicities were observed and although no objective response were observed in lymphoid malignancies, four or six patients with CLL had a reduction in lymphocytosis or lymphadenopathy. A recently Japanese phase I study evaluating everolimus in relapsed or refractory NHL confirmed no dose-limiting toxicities. Witzig and colleagues recently published a phase II study in which everolimus was tested in relapsed lymphoma, with an ORR of 30% with no major differences between DLBCL, MCL and FL. In relapsed/refractory WM, Ghobrial and colleagues demonstrated an ORR of 70% (42% PR).

In conclusion, rapalogs show a certain degree of activity in relapsed/refractory NHL; however, with the exception of WM, a relatively low percentage of complete and durable responses has been reported. For these reasons, new mTOR inhibitor molecules are under evaluation and in particular the association with other classes of drugs is warranted to increase their efficacy.

CAL-101. The PI3K/AKT/mTOR pathway is essential in the survival of several different B-cell NHLs and therefore represents an attractive therapeutic target. The most important member in upstream part of this pathway is the

p110d isoform of PI3K, which is restricted to to cells of haematopoietic origin. CAL-101 is an oral potent p110d-selective PI3K inhibitor. Kahl and colleagues reported a phase I trial with this agent in 56 patients with an ORR of 62%. Notably, the most important dose-limiting toxicity was represented by reversible abnormalities in transaminases (33%). Further studies are planned for combinations of this with other agents including rituximab and bendamustine. [4]

BTK inhibitors

Burton's tyrosine kinase (BTK) is a key component of the BCR signalling pathway. The orally bioavailable compound PCI-32765 is a selective and permanent inhibitor of BTK, resulting in block of BCR-stimulated activation of NF-kB and ERK, inhibition of growth and induction of apoptosis of B cells. Fowler and colleagues presented the results of an ongoing phase I trial of PCI-32765 in relapse/refractory B-cell malignancies. In 35 patients with iNHL, ORR was 40% (1 CR and 13 PR) and responses weredetected across all histologic subtypes and were quite durable.Side-effects were mild, with few grade 3 (9/47) and no grade 4 toxicities at this point in the dose escalation.

BCL-2 inhibitors

The Bcl-2 family is a group of proteins that may be either anti-apoptotic (e.g. Bcl-2, Bcl-X) or pro-apoptotic (Bax, Bak). The t(14;18), which occurs in majority of FL, results in the juxta-position of the BCL-2 gene next to the immunoglobulin heavy chain gene, leading to its constitutive expression and to signalling unbalance in favor of survival of malignant cells. AT-101, an orally

active Bcl-2 inhibitor, demonstrated some activity in patients with CLL, but was associated with dose-limiting hepatic and gastrointestinal toxicity. The orally available Bcl-Xl-inhibitor ABT-263 showed activity primarily in CLL, with thrombocytopenia being the main toxicity. Obatoclax is a novel Bcl-2 inhibitor that appear to sensitize rituximab-resistant lymphoma cells to treatment with bortezomib; this agent is currently undergoing clinical investigation in combination with other agents in FL.

Agents that target the microenvironment

Lenalidomide

Lenalidomide is an immunomodulatory drug (IMiD) which is 10,000 times more potent and displays a better safety profile than its parent compound thalidomide. The key to the therapeutic potential of lenalidomide lies in the fact that it has multiple mechanisms of action, resulting in anti-inflammatory, anti-angiogenic, and antitumor effects in a wide spectrum of hematological malignancies, such as myelodisplastic syndromes, multiple myeloma (MM) and B-cell NHL. To date, linalidomide has been associated with TNF-alfa inhibitory, T-cell costimulatory and anti-angiogenic effects. With respect to thalidomide, lenalidomide displays single-agent better efficacy, has a better safety profile and does not cause significant somnolence, constipation or peripheral neuropathy. However, myelosuppression can be a serious problem, and frequently a reduction of doses and/or granulocyte growth factor support is required. Both lenalidomide and thalidomide have comparable incidences of venous thrombotic disease (deep vein thrombosis or pulmonary embolism).

Lenalidomide has shown promising results in the tratment of patients with NHL. In a perspective phase II study evaluating the safety and the efficacy of lenalidomide monotherapy in 43 patients with relapsed/refractory iNHL the ORR was only 23% (3 CR and 7 PR); however responses were quite durable (more than 16 months). The median time of antitumor response was 3.6 months and the median PFS for the whole group was 4.4 months. Adverse events were predictable and manageable; the most common grade 3-4 adverse events were neutropenia (46%) and thrombocytopenia (19%).

Based on preclinical studies demonstrating synergistic activity between rituximab and linalidomide in lymphoma models, several investigators are evaluating the efficacy and the tolerability of lenalidomide in combination with rituximab in iNHL. Results of a randomized CALGB study, evaluating lenalidomide alone versus lenalidomide and rituximab (RR or R2 regimen) in 94 patients with recurrent FL (previously exposed to rituximab), were presented at the last ASCO meeting (2012). Briefly, RR was more active than lenalidomide alone and demonstrate significantly longer EFS, with similar hematologic toxicity. Recently, Fowler and colleagues reported the preliminary results of treatment with RR in newly diagnosed iNHL patients (n = 48; 30 FL). The ORR was 86% for the entire cohort (CR 79%) and 93% for FL patients (CR 86%). At a median follow up of 20 months PFS was 91%. Adverse events were similar to those observed in lenalidomide single-agent studies. Of interest, no significant tumor-flare reactions were observed. Results of currently ongoing randomized trial evaluating the activity of this chemotherapy-free regimen in comparison with the current standard immunochemotherapy in iNHL are eagerly awaited. [4]

Aim

The main purpose of this work was understanding how to treat Splenic Marginal Zone Lymphoma (SMZL) in patients unfit for surgery or more aggressive therapies.

Methods

30 newly diagnosed SMZL patients were included in this analysis.

We enrolled all cases, consecutively treated in our Institution, not eligible for Rituximab-Cladribrine regimen or more toxic schedules because of comorbidity or previous serious infective episodes.

Eligibility criteria were: histological diagnosis of SMZL, age >18 years, HIV negativity, two or more signs of active disease (symptomatic splenomegaly, constitutional symptoms , severe peripheral cytopenias); written informed consent.

Patient evaluation included a full history and clinical examination, complete serum biochemistry with dosage of LDH and β2microglubulin, peripheral blood and bone marrow immunophenotyping, bone marrow biopsy, bone marrow molecular analysis, chest and abdomen and pelvic computed tomographic (CT) scan, serology for HIV, HBV and HCV.

Diagnosis was based on lymphocyte morphology, immunophenotype of peripheral blood and bone marrow samples, bone marrow biopsy and spleen histology when available.

Patients'clinical characteristics are shown in Table 1. One case was HCV-positive.

According to IIL prognostic index, 11 patients resulted in low-risk group, 10 patients in the intermediate group and 9 cases in the high risk group.

Sex	Male: 20 Female: 10 Total: 30
Median Age	70 years (range 59-82)
ECOG at diagnosis	Numbers of patients
0	8
1	8
2	14
3, 4, 5	0
Median LDH at diagnosis	321 U/L (range 143 U/L-704 U/L)
Median Hb at diagnosis	10,7 gr/dL (range 7,2gr/dL -15,4 gr/dL)
Median Albumine at diagnosis	4,069 gr/dL (range 4,89gr/dL -2,68 gr/dL)
Stage	Numbers of patients
I	3
II	2
III	1
IV	23
Not Avialable	1
IPI	Numbers of patients
0	0
1	5
2	12
3	11
4	0
5	0
Not Avialable	2
IIL score prognostic inedx	Numbers of patients
Low	11
Int	10
High	9
Toxicity	Numbers of patients
Grade I anemia	3
Grade I neutropenia	1

Tab. 1

Treatment

Cyclophosphamide was assumed at a dose of 100 mg per day for 15 consecutive days. Cycles were repeated every 30 days for a total of six cycles. Rituximab was administered iv at a dose of 375 mg/m^2 on day 8 of each cycle. Rituximab was infused over a 3-6 hour period, on an outpatient basis. Patients were pre-medicated with diphenhydramine (40 mg orally) and acetominophen (1 g orally).

Patients were evaluated for response 2 months after the end of treatment, then every 3 months during the first 2 years and every 6 months for further 3 years. Bone marrow samples and bone marrow biopsy were performed in all patients at the end of therapy.

Response to therapy was evaluated according to Cheson revised criteria (2007). CTCAE v.4 criteria were used to assess toxicity.

Statistical analyses

All calculations were performed using the SPSS for Windows, release *21, 2012*. Overall survival and time to progression (PFS) were estimated using the Kaplan-Meier test. PFS as computed from the beginning of treatment to further disease progression, relapse or death.

Results

Between October 2005 and October 2012, 30 patients (20 male and 10 female; median age 70 years, range 51-83) with newly diagnosed SMZL were enrolled in this study.

All patients were evaluable for clinical response. Overall response rate (ORR) was 87%: 21/30 (70%) achieved a complete hematological response (CR), 5/30 (17%) a partial response (PR), and 4/30 (13%) resulted unresponsive.

The median reduction of spleen size was 4 cm (range, 3-6 cm) among the cases that achieved a CR.

After a median follow-up was 25 months (range 6-67), 2 out of 26 responsive cases relapsed with a median PFS of 20 months (range, 1-53).

IIL prognostic index score influenced significantly PFS with a better outcome for low risk patients as demonstrated by PFS Kaplan Maier curves (no censored data were observed in low risk patients curve) and log-rank test of Mantel-Cox (P=0.025): median PFS was 24 months and 19 months respectively for low-risk group and intermediate/high risk one (fig.1).

All patients were evaluable for toxicity. Globally, this regimen was well tolerated and it was not necessary nor discontinue therapy or delay it in any case. Infectious events were not recorded. Haematological toxicity was mild (neutropenia or anemia of grade I).

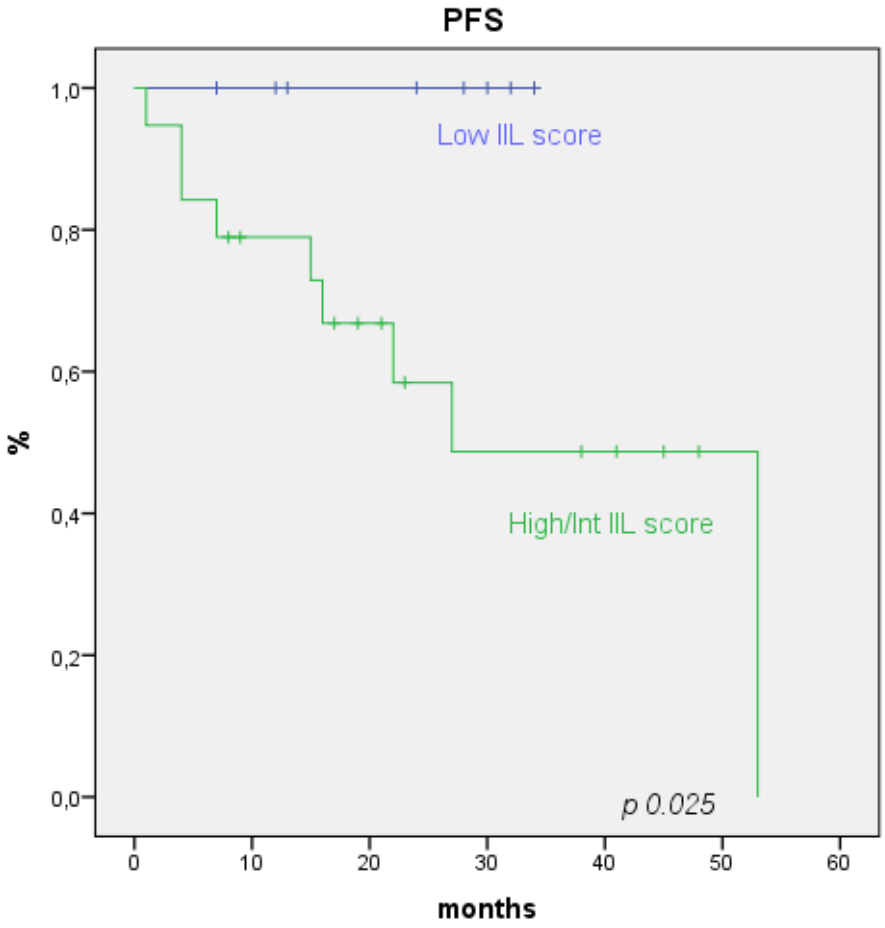


Fig.1: IIL prognostic index score influenced significantly PFS with a better outcome for low risk patients (no censored data were observed in low risk patients curve): median PFS was 24 months and 19 months respectively for low-risk group and intermediate/high risk one (P=0,025).

Discussion

Splenic marginal lymphoma usually presents as an indolent disease that generally affects older patients, and that because of its clinical features often does not need to be treated [5-7].
Where symptoms appear , the choice of treatment depends on the clinical localization, presence of comorbidities, performance status and age of patient. The therapeutic approach (surgical, immunotherapy, immunochemotherapy) should be defined without forgetting that it is an "indolent" disease and that the quality of life, especially in very elderly patients, is one of the most important objective [8-10].
In this light, especially in cases not eligible for surgery, or in patients where the main purpose of the treatment is the control of the symptomatology and not an improvement of survival curves, the use of oral drugs, could be a valid choice. In this context, alkylating agents such as chlorambucil and cyclophosphamide seem to have an interest role [23].
The advent of Rituximab has dramatically changed the outcome of all lymphomas, even of marginal histotypes [16]. To date there are many data demonstrating its efficacy as monotherapy and in combination in these kind of patients [16, 24-26].
The present study was designed with the aim to evaluate the activity of oral cyclophosphamide associated with rituximab as first line therapy in patients with SMZL unfit for more aggressive therapies. Thirty patients were evaluated. ORR resulted 87% . IIL score prognostic index evidenced better outcome for low score patients with median PFS of 24 months versus 19 months of high and intermediate risk ones (P =0,025). No significant adverse event were

recorded. Comparing these results with those obtained on 20 cases with similar clinical features treated with rituximab alone in our center (unpublished data) , there was an advantage in terms of percentage of responses (87% versus 63%) for the group treated with the combination Rituximab-oral cyclophosphamide.

Therefore, this therapeutic approach has proven effective enough and with a good toxicity profile. The impact on quality of life was in fact minimal, ensuring in most cases a normal lifestyle.

Based on these data, although the small cohort of patients, we believe that this schedule could be considered in SMZL patients, requiring therapy but who cannot sustain approaches potentially more toxic.

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